

Clinical Laboratory Improvement Advisory Committee

FDA White Oak Campus

Tuesday, April 10, 2018

Implementation of Next-Generation Sequencing in Clinical and Public Health Laboratories

Background/Introduction

Ira M. Lubin, PhD, FACMG

Geneticist

Quality and Safety Systems Branch

Implementation of Next Generation Sequencing in Clinical Laboratories

- **Background / Introduction** **Ira M. Lubin, PhD, FACMG**
- **Public Health Perspective** **Rebecca Hutchins, MS, MBA**
- **Clinical Laboratory Perspective** **John D. Pfeifer, MD, PhD**
- **CLIAC Discussion**

Background / Introduction

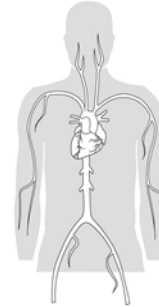
- **Next-generation sequencing in clinical and public health applications**
- **Integrating next-generation sequencing into clinical laboratory testing**
- **Practice and regulatory framework for assuring the quality of clinical next-generation sequencing**
- **Proposed CLIAC Workgroup**
- **Questions for CLIAC**

Next-Generation Sequencing in Clinical and Public Health Applications

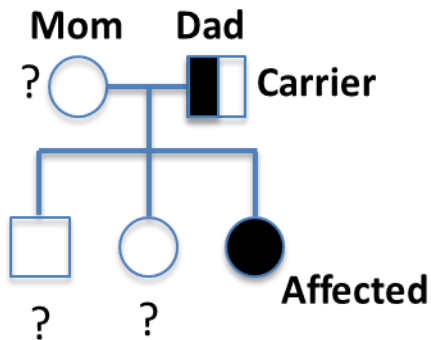


Pediatrics:

- Rare Disease
- Developmental Disabilities

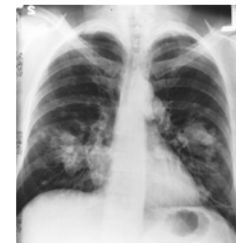


Chronic Disease



Screening:

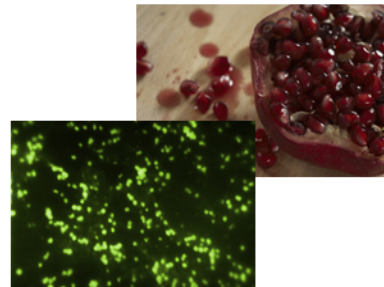
- Carrier Screening



Cancer



Transplant



Pathogens:

- Identification
- Outbreak response
- Antibiotic resistance

What is the Landscape of Next-Generation Sequencing Offerings in the United States?

New York Clinical Laboratory Evaluation Program

<https://www.wadsworth.org/regulatory/clep/approved-ldt>

81 Laboratories

(searched 3/23/2018)

College of American Pathologists Accreditation Data (per organizational contact)

331 laboratories

(provided 3/16/2018)

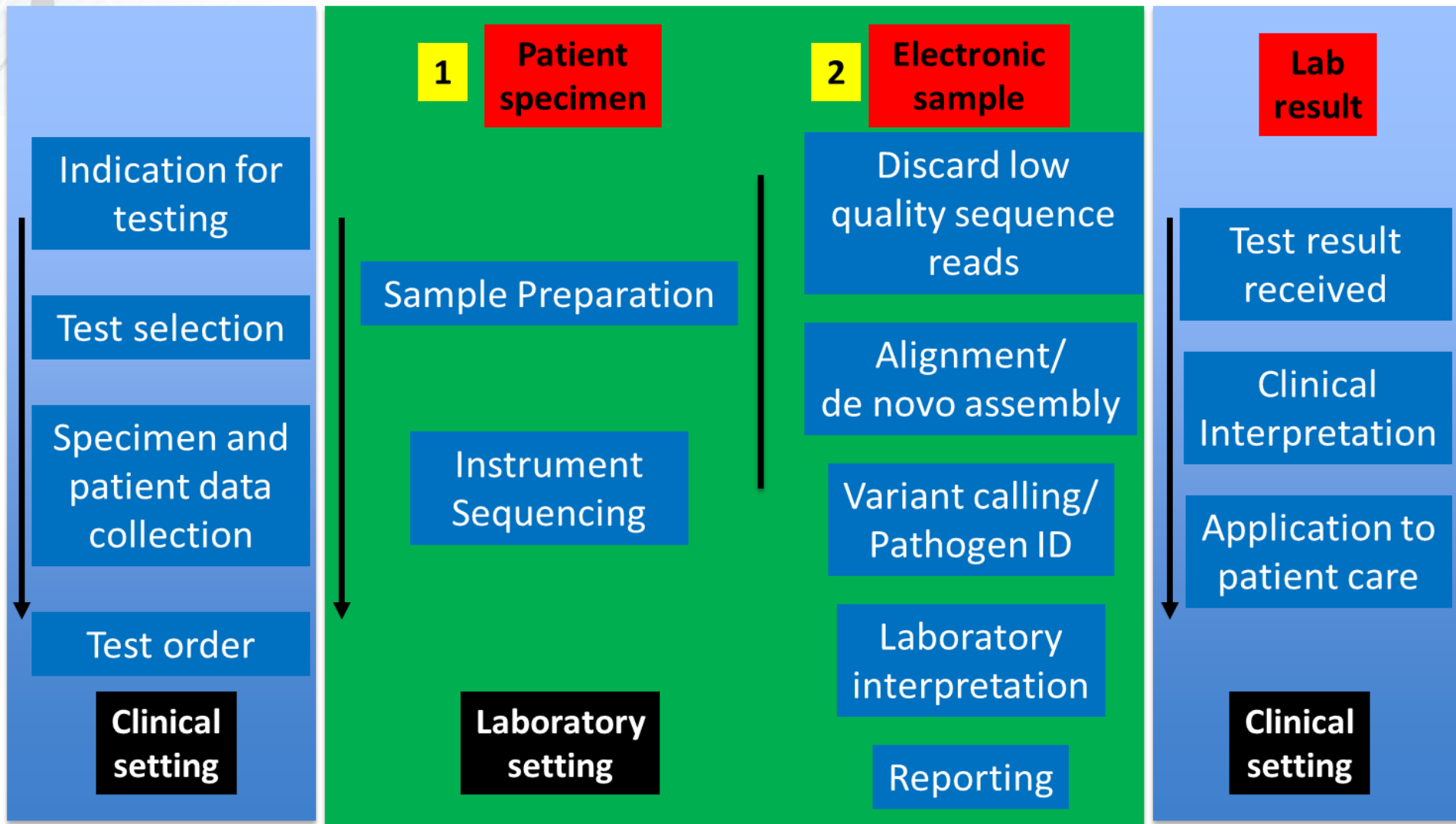
Genetic Testing Registry

<https://www.ncbi.nlm.nih.gov/gtr/>

80 Laboratories

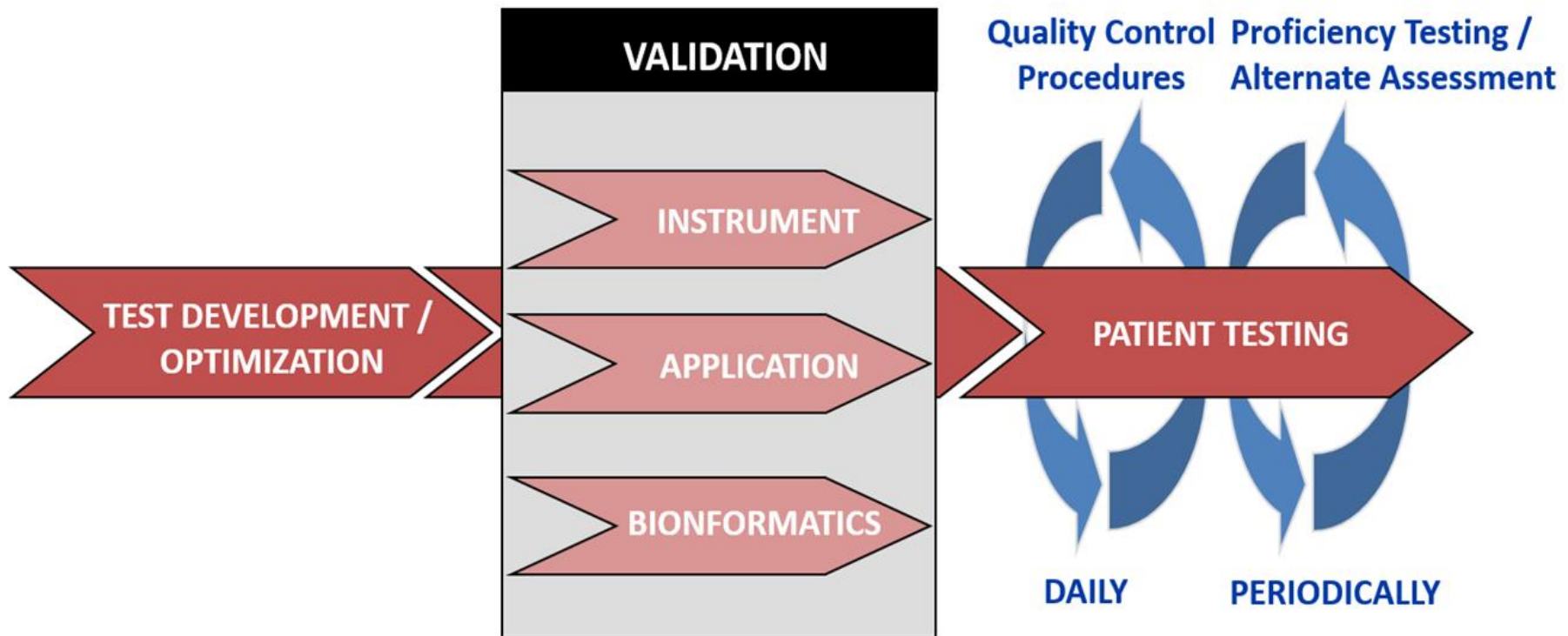
(Provided 3/12/2018)

Workflow: Next Generation Sequencing



Framework for the Implementation of Clinical NGS Testing

As of 2018, the majority of clinical NGS tests are developed within the laboratory that they are offered.



Gargis et al., Nat Biotechnol. 2012;30:1033

Practice and Regulatory Framework for Assuring the Quality of Clinical Next-Generation Sequencing

Applying the CLIA regulations to clinical next-generation sequencing is challenging due to the novelty and complexity of the technology, and new paradigms for data analyses

- **Code of Federal Regulations. The Clinical Laboratory Improvement Amendments (CLIA).** 42 CFR Part 493 sec. 1256.
- **Interpretive guidelines:** https://www.cms.gov/Regulations-and-Guidance/Guidance/Manuals/Downloads/som107ap_c_lab.pdf

Applying CLIA to Next-Generation Sequencing in the Clinical Setting

- Test validation (*CLIA general requirements at §493.1253*)
- Quality control procedures (*CLIA general requirements at §493.1256; unidirectional workflow for molecular amplification at §493.1101 (a)(3)*)
 - Quality control materials (physical and electronic)?
 - Use of external data to support analyses?
- Test ordering (*CLIA general requirements at §493.1241*)
- Result Reporting (*CLIA general requirements at §493.1291*)
- Proficiency testing (*CLIA does not specify PT requirements for NGS, so alternative performance assessment requirements at §493.1236(c) apply*)
- Personnel competencies (*Subpart M; No specific requirements*)
- Bioinformatics pipeline quality assessment (*No specific requirements*)

Other issues:

- Distributive testing – single test / multiple sites
- Reporting of secondary findings
- Reanalysis

Next Generation Sequencing: Standardization of Clinical Testing

Assuring the quality of next-generation sequencing in clinical laboratory practice

To the Editor:

We direct your readers' attention to the principles and guidelines (Supplementary Guidelines) developed by the Next-generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) workgroup. These guidelines represent initial steps to ensure that results from tests based on next-generation sequencing (NGS) are reliable and useful for clinical decision making. The US Centers for Disease Control and Prevention (CDC) convened this national workgroup, which collaborated to define platform-independent approaches for establishing technical process elements of a quality management system (QMS) to assure the analytical validity and compliance of NGS tests with existing regulatory and professional quality standards. The workgroup identified and addressed gaps in

The workgroup recommendations are summarized in Table 1. Although the workgroup focused on detection of DNA sequence variations associated with heritable human disorders, many of the principles and recommendations described are also relevant to the application of NGS to other areas of laboratory medicine, including the diagnosis, prognosis and

treatment of cancer and infectious-disease testing.

Validation is the process of establishing analytical performance specifications for a clinical test system developed in house to confirm that the system is suitable for its intended use¹. During the validation process, the laboratory must demonstrate that the assay functions as expected and provides

Table 1 Selected workgroup recommendations for establishing NGS test clinical use

Requirements for test establishment	Objective	NGS-specific recommendations*
Validation	Document reliability of the platform, test, and informatics pipeline before testing of patient specimens	<ul style="list-style-type: none"> Platform validation: establish that the system produces accurate sequence analysis across the genomic regions targeted for testing. Test validation: establish that the system correctly identifies disease-associated (and other) variants in target genome (Supplementary Guidelines, section 4). Informatics pipeline validation: establish that the pipeline reliably analyzes platform data to produce an accurate

Gargis et al. Nat. Biotechnol. 2012; 30:1033-1036+ Supplemental

Principles and Recommendations for Standardizing the Use of the Next-Generation Sequencing Variant File in Clinical Settings

Ira M. Lubin,^{*} Nazneen Aziz,^{††} Lawrence J. Babb,[§] Dennis Ballinger,[¶] Himani Bisht,^{||} Deanna M. Church,^{**††} Shaun Cordes,[¶] Karen Eilbeck,^{††} Fiona Hyland,^{§§} Lisa Kalman,^{*} Melissa Landrum,^{††} Edward R. Lockhart,^{*} Donna Maglott,^{††} Gabor Marth,^{¶¶} John D. Pfeifer,^{||||} Heidi L. Rehm,^{***} Somak Roy,^{†††} Zivana Tezak,^{||} Rebecca Truty,[¶] Mollie Ullman-Cullere,^{†††} Karl V. Voelkerding,^{§§§} Elizabeth Worthey,^{***} Alexander W. Zaranek,^{|||||} and Justin M. Zook^{††††}

Good laboratory practice for clinical next-generation sequencing informatics pipelines

To the Editor:

We report principles and guidelines (Supplementary Note) that were developed by the Next-Generation Sequencing: Standardization of Clinical Testing II (Nex-StoCT II) informatics workgroup, which was first convened on October 11–12, 2012, in Atlanta, Georgia, by the US Centers for Disease Control and Prevention (CDC; Atlanta, GA). We present

recommendations are summarized in Table 1, and detailed in the guidelines presented in the Supplementary Note.

Currently, most clinical NGS tests are offered as laboratory-developed tests (LDTs), which are tests designed, manufactured and used within a single laboratory. These tests use commercially available sequencing platforms to generate raw sequence data that are subsequently

Lubin IM et al. J Mol Diagn. 2017;19:417-426

Gargis et al. Nat. Biotechnol. 2015;33: 689-693 + Supplemental

CDC facilitated the development of the first consensus guideline for clinical next-generation sequencing.

The Food and Drug Administration: Draft Guidance for Next-Generation Sequencing

Contains Nonbinding Recommendations

Draft - Not for Implementation

Infectious Disease Next Generation Sequencing Based Diagnostic Devices: Microbial Identification and Detection of Antimicrobial Resistance and Virulence Markers

Draft Guidance for Industry and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

Document issued on: May 13, 2016

Contains Nonbinding Recommendations
Draft - Not For Implementation

Use of Standards in FDA Regulatory Oversight of Next Generation Sequencing (NGS)-Based In Vitro Diagnostics (IVDs) Used for Diagnosing Germline Diseases

Draft Guidance for Stakeholders and Food and Drug Administration Staff

DRAFT GUIDANCE

This draft guidance document is being distributed for comment purposes only.

Document issued on July 8, 2016.

You should submit comments and suggestions regarding this draft document within 90 days of

Contains Nonbinding Recommendations
Draft - Not For Implementation

Use of Public Human Genetic Variant Databases to Support Clinical Validity for Next Generation Sequencing (NGS)-Based In Vitro Diagnostics

Draft Guidance for Stakeholders and Food and Drug Administration Staff

DRAFT GUIDANCE

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Document issued on July 8, 2016.

You should submit comments and suggestions regarding this draft document within 90 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to <http://www.regulations.gov>. Submit written

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February 2014

MMoG-A2

Nucleic Acid Sequencing Methods in Diagnostic Laboratory Medicine; Approved Guideline—Second Edition

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ACMG PRACTICE GUIDELINES **Genetics in Medicine**

ACMG clinical laboratory standards for next-generation sequencing

Heidi L. Rehm, PhD^{1,2}, Sherri J. Bale, PhD³, Pinar Bayrak-Toydemir, MD, PhD⁴, Jonathan S. Berg, MD⁵, Kerry K. Brown, PhD⁶, Joshua L. Deignan, PhD⁷, Michael J. Friez, PhD⁸, Birgit H. Funke, PhD⁹, Madhuri R. Hegde, PhD¹⁰ and Elaine Lyon, PhD¹¹, for the Working Group of the American College of Medical Genetics and Genomics Laboratory Quality Assurance Committee

Disclaimer: These American College of Medical Genetics and Genomics (ACMG) clinical laboratory standards are not intended to be used as a sole basis for clinical decision-making. They do not necessarily ensure a successful medical outcome. They are intended to provide a framework for clinical decision-making. They are not intended to be used as a sole basis for clinical decision-making. They are not intended to be used as a sole basis for clinical decision-making.

Next-generation sequencing technologies have been deployed in clinical laboratories, enabling rapid and accurate diagnosis. These technologies have large-scale sequencing by several orders of magnitude and are being made available to a wide range of clinical laboratories. Next-generation sequencing technologies are also facilitating further advances in clinical decision-making and disease prevention for at-risk individuals with rapid advances and additional challenges in validation and use of these constantly evolving technologies.

A. INTRODUCTION

Sequencing technologies have evolved rapidly over the past few years. Semi-automated Sanger sequencing is standard for many years and is still in use. However, its limitations include low throughput, making multiple panels have relatively high cost. Next-generation sequencing technologies have evolved rapidly over the past few years. Semi-automated Sanger sequencing is standard for many years and is still in use. However, its limitations include low throughput, making multiple panels have relatively high cost. Next-generation sequencing technologies have evolved rapidly over the past few years. Semi-automated Sanger sequencing is standard for many years and is still in use. However, its limitations include low throughput, making multiple panels have relatively high cost.

Genetics in Medicine | Volume 15 | Number 3 | September 2013

EUROPEAN JOURNAL OF HUMAN GENETICS 24, 2–5
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POLICY

Guidelines for diagnostic next-generation sequencing

Gert Matthijs^{1,2}, Erika Soucek^{1,2}, Mariëtte Alders^{1,2}, Annick Corveleyn¹, Sebastian Eck¹, Ilse Feenstra¹, Valérie Raco¹, Erik Stiermans¹, Marc Sturm¹, Marjan Weiss¹, Hedy Yntema¹, Eibert Bakker¹, Hans Scheffer¹ and Peter Bauer³

We present, on behalf of EuroGenTest and the European Society of Human Genetics, guidelines for the evaluation and validation of next-generation sequencing (NGS) applications for the diagnosis of genetic disorders. The work was performed by a group of laboratory geneticists and bioinformaticians, and discussed with clinical geneticists, industry and patients' representatives, and other stakeholders in the field of human genetics. The statements that were written during the elaboration of the guidelines are presented here. The background document and full guidelines are available as supplementary material. They include many examples to assist the laboratories in the implementation of NGS and accreditation of this service. The work and ideas presented by others in guidelines that have emerged elsewhere in the course of the past few years were also considered and are acknowledged in the full text. Interestingly, a few new insights that have not been cited before have emerged during the preparation of the guidelines. The most important new feature is the presentation of a 'testing system' for NGS-based diagnostic tests. The guidelines and statements have been approved by the genetic diagnostic community, and thus seem to be valuable for the harmonization and quality assurance of NGS diagnostics in Europe.

European Journal of Human Genetics (2014) 24, 2–5; doi:10.1038/ejhg.2015.226; published online 28 October 2015

Next-generation sequencing (NGS) allows for the fast generation of thousands to millions of base pairs of DNA sequence of an individual patient. The relatively fast emergence and the great success of these technologies in research hold a new era in genetic diagnostics. However, the new technologies bring challenges, both at the technical level and in terms of data management, as well as for the interpretation of the results and for counseling. We believe that all these aspects warrant consideration of what the precise role of NGS in diagnostics will be, and in terms of data management, as well as for the interpretation of the results and for counseling. We believe that all these aspects warrant consideration of what the precise role of NGS in diagnostics will be, and in terms of data management, as well as for the interpretation of the results and for counseling.

College of American Pathologists' Laboratory Standards for Next-Generation Sequencing Clinical Tests

Nazim Azir, PhD, Qin Zhao, PhD, Lynn Bry, MD, PhD, Denise K. Driscoll, MS, MT/ASCP/SBB, Birgit Funke, PhD, Jane S. Gibson, PhD, Wayne W. Grody, MD, Madhuri R. Hegde, MD, Gerald A. Hodge, MD, Debra C. B. Leonard, MD, PhD, Jason D. Meeker, MD, PhD, Rakesh Nagarajan, MD, PhD, Linda A. Palko, MT/ASCP, Ryan S. Rabinovitz, MD, Im Schjerve, MD, Karen E. Weick, MD, Karl V. Voelkeling, MD

Context:—The higher throughput and lower per-base cost of next-generation sequencing (NGS) as compared to Sanger sequencing has led to its rapid adoption in clinical testing. The number of laboratories offering NGS-based tests has also grown considerably in the past few years, despite the fact that specific Clinical Laboratory Improvement Amendments (CLIA) of 1988/Clinical Laboratory Improvement Programs (CLIP) laboratory standards had not yet been developed to regulate this technology.

Objective:—To develop a checklist for clinical testing using NGS technology that sets standards for the analytic wet bench process and for bioinformatics or "dry bench" analyses. As NGS-based clinical tests are new to diagnostic testing and are of much greater complexity than traditional Sanger sequencing-based tests, there is an urgent need to develop new regulatory standards for laboratories offering these tests.

Design:—To develop the necessary regulatory framework for NGS and to facilitate appropriate adoption of this technology for clinical testing, CAP formed a committee in 2011, the NGS Work Group, to deliberate upon the contents to be included in the checklist.

Results:—A total of 18 laboratory accreditation checklist requirements for the analytic wet bench process and bioinformatics analysis process have been included within CAP's molecular pathology checklist (MOL).

Conclusions:—This report describes the important issues considered by the CAP committee during the development of the new checklist requirements, which address documentation, validation, quality assurance, confirmatory testing, exception logs, monitoring of assays, variant interpretation and reporting, incidental findings, data storage, version traceability, and data transfer confidentiality.

Arch Pathol Lab Med 2013;137:481–493; doi:10.5858/arpa.2014-0250-CF

DNA sequencing has evolved from Maxam-Gilbert¹ and Sanger^{2,3} methods in the 1970s to a set of technologies that are collectively referred to as next-generation sequencing

The Journal of Molecular Diagnostics, Vol. 17, No. 4, November 2015

ELSEVIER

SPECIAL ARTICLE

Next-Generation Sequencing for Infectious Disease Diagnosis and Management

A Report of the Association for Molecular Pathology

Martina I. Lefterova,^{1,2} Carlos J. Suarez,^{1,2} Niaz Banat,^{1,2} and Benjamin A. Pinsky^{1,2}

From the Association for Molecular Pathology Next-Generation Sequencing in Infectious Disease Work Group,¹ Bethesda, Maryland, and Pathology² and the Department of Medicine,³ Division of Infectious Diseases and Geographic Medicine, Stanford University School of Medicine, Stanford, California

ACMG Accreditation Statement: This activity ("2015 CME Program in Molecular Diagnostics") has been planned and implemented in accordance with the Accreditation Council for Continuing Medical Education (ACCME) through the joint sponsorship of the American Society for Clinical American Society for Investigative Pathology (ASIP). ASIP is accredited by the ACCME to provide continuing medical education for physicians.

The ASIP designates this journal-based CME activity ("2015 CME Program in Molecular Diagnostics") for a maximum of 36 ASIP PRA Group credit only claim credit commensurate with the extent of their participation in the activity.

ACMG Disclosure: The authors of this article and the planning committee members and staff have no relevant financial relationships with commercial entities.

Accepted for publication July 2, 2015.

Address correspondence to Benjamin A. Pinsky, M.D., Ph.D., Department of Pathology, Stanford University School of Medicine, 3075 Hillview Ave, Room 213, Palo Alto, CA 94304. E-mail: bpinsky@stanford.edu

Next-generation sequencing (NGS) technologies are increasingly being used for diagnosis of infectious diseases. Herein, we review the application of NGS in clinical microbiology, resistance testing, direct detection of unknown disease-associated pathogens, investigation of microbial population diversity in the human host, and strain typing, review into three main sections: (i) applications in clinical virology, (ii) applications in mycobacteriology, and (iii) validation, quality control, and maintenance. Although NGS holds enormous promise for clinical infectious disease testing, including automation, standardizing technical protocols and bioinformatics pipelines, establishing proficiency testing and quality control measures, and reducing time, all of which would be necessary for widespread adoption of NGS in clinical microbiology laboratories.

J Mol Diagn 2015; 17: 623–634; doi:10.1016/j.jmoldi.2015.07.004

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Best practices for evaluating single nucleotide variant calling methods for microbial genomics

Nathan D. Olson^{1,2}, Steven P. Lund³, Rebecca E. Colman⁴, Jeffrey T. Foster⁴, Jason W. Sahi^{1,2}, James M. Schupp^{1,2}, Paul Keim^{1,2}, Javne B. Morrow¹, Marc L. Salt^{1,2} and Justin M. Zook¹

¹Bioreactors and Biomaterials Division, Material Measurement Lab, Gaithersburg, MD, USA; ²Statistical Engineering Division, Informatics and Technology, Gaithersburg, MD, USA; ³Division of Pathogen Prevention, Flagstaff, AZ, USA; ⁴Center for Microbial Genomics and Genomics, USA; ⁵Department of Bioengineering, Stanford University, Stanford, CA, USA

Innovations in sequencing technologies have advanced in understanding biological systems increasingly recognize that analyzing the vast

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SALLY DRESLIN, M.S., R.N. Executive Deputy Commissioner

UPDATED AND REVISED

Oncology—Molecular and Cellular Tumor Markers

"Next Generation" Sequencing (NGS) guidelines for somatic genetic variant detection

The following describes requirements for the development of procedures and the establishment of performance (validation) of assays for the detection of somatic genetic variants by Next

Applications of Clinical Microbial Next-Generation Sequencing

A report from the AMERICAN ACADEMY OF MICROBIOLOGY

SPECIAL ARTICLE

Guidelines for Validation of Next-Generation Sequencing—Based Oncology Panels

A Joint Consensus Recommendation of the Association for Molecular Pathology and College of American Pathologists

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¹Validation Working Group of the Clinical Practice Committee, ²Association for Molecular Pathology¹ Children's Hospital of Chicago,³ Northwestern University's Feinberg School of Medicine, Chicago, Illinois; ⁴New York, New York; the Department of Pathology and Knight Cancer Institute,⁵ Oregon Health and Science Clinical Laboratory Genetics, University Health Network, Toronto, Ontario, Canada; the Department of Pathology, University of Toronto, Toronto, Ontario, Canada; the Centers for Disease Control and Prevention,⁶ Atlanta, Georgia

Summary

- **Next generation sequencing is continuing to integrate into diverse medical disciplines.**
- **Bioinformatics (computational analysis) comprises a significant component of the test.**
- **Communication between the laboratory professional (s) and clinician(s) is vital toward understanding of the available testing, limitations, and test findings**
- **Clinical NGS testing is covered under CLIA but application of the laboratory requirements may not be sufficiently defined.**

Proposed CLIAC NGS Workgroup

Proposed Charge

Provide input to CLIAC for consideration in developing recommendations to CDC, CMS, and FDA for assuring the quality of next generation sequencing in clinical laboratory settings

Proposed Workgroup Tasks

- **Identify challenges in applying the existing regulatory framework**
- **Identify challenges and gaps in guidance**
- **Consider and suggest strategies to address the identified gaps and challenges**
- **Consider and suggest strategies for assuring workforce competency**

Questions for CLIAC

- 1. What major gaps and issues do laboratories experience when implementing NGS-based testing for various applications?**
- 2. What guidance is lacking for laboratories that perform NGS testing to address critical steps in the total testing process?**
- 3. How can CDC, CMS, and FDA assist in filling NGS-related gaps and providing guidance to laboratories?**



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For more information please contact Centers for Disease Control and Prevention

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.